REMARKS

The Present Invention and Pending Claims

Claims 1-12 are pending and directed to a stable composition comprising a PQQ-dependent glucose dehydrogenase (claims 1, 2, and 5-8) and a method of preparing the composition (claims 3, 4, and 9-12). The composition of the present invention has a much higher specific activity relative to the total weight of the composition than other such compositions known in the art. Additionally, the amount of stabilizing agent contained in the composition of the present invention is remarkably low. These characteristics have several advantages, including the reduction of a potential adverse effect on an enzyme reaction by impurities which may be contained in the stabilizing agent, and the reduction of errors in measuring small amounts of solution with pipettes and the like, as a result of the low viscosity of solution due to the low concentration of a stabilizing agent. Moreover, since the enzyme composition of the present invention is primarily intended to be utilized as a sensor of blood glucose, whereby the enzyme composition is applied to a sensor tip, the high specific activity of the composition means that only a small amount of the composition is needed for application, which assists in the reduction of scatter in the measured values.

Amendments to the Claims

The claims have been amended to claim more distinctly and point out more particularly the present invention. Specifically, claims 6-8 and 10-12 have been amended to replace "derived from" with "obtained from" as suggested by the Office, and to italicize the terms "*Acinetobactor*" and "*Acinetobactor calcoaceticus*." Claims 7, 8, 11, and 12 have been amended to correct the spelling of "*Acinetobactor*." Additionally, claims 8 and 12 have been amended to correct the spelling of "NCIMB 11517." Accordingly, no new matter has been presented by way of these amendments.

The Office Action

The Office has rejected claims 6-12 under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Claims 8 and 12 have been rejected under 35 U.S.C. § 112, first paragraph, for alleged lack of enablement. The Office has reiterated the rejection of claims 1-12 under 35 U.S.C. § 103(a) as allegedly obvious over Sode et al. (*Biotechnology Techniques, 11*(8), 577-580 (1997)) in view of Adachi et al. (JP 09-140378). Reconsideration of these rejections is hereby requested.

Discussion of the Section 112, Second Paragraph, Rejection

The Office has rejected claims 6-12 for the recitation of "derived from," because the phrase is allegedly indefinite. Applicants have amended the pending claims to replace "derived from" with "obtained from" as suggested by the Office. Accordingly, this rejection is believed to be moot and should be withdrawn.

Discussion of the Section 112, First Paragraph, Rejection

The Office contends that a deposit of *Acinetobactor calcoaceticus* NCIMB 11517, which is recited in claims 8 and 12, is required because it is not clear if the written description is sufficiently repeatable to avoid the need for such a deposit. Applicants respectfully disagree that such a deposit is necessary.

Acinetobactor calcoaceticus NCIMB 11517 is available from the National Collections of Industrial, Food and Marine Bacteria (NCIMB), which widely provides preservation and supply services of strains. NCIMB is an internationally recognized agency of Scotland, UK, such that an ordinarily skilled artisan would understand that "*Acinetobactor calcoaceticus* NCIMB 11517" indicates the availability of the strain from NCIMB. Thus, the strain recited in claims 8 and 12 was, at the time of the filing of the application, and is today, readily available from the NCIMB. Moreover, there is no reason to believe that the availability of the strain recited in claims 8 and 12 will be impaired in the future.

In that the strain is available from the NCIMB, and there is no reason to believe that the strain will not be available in the future, there is no need for applicants to deposit such a strain. As a result, the rejection should be withdrawn. Please note that the species name of the strain was changed to *Acinetobacter baumannii* NCIMB 11517 subsequent to the filing of the application, but that the change in name does not affect the availability of the strain.

Discussion of the Section 103(a) Rejection

The rejection of the pending claims in view of the Adachi and Sode references has been maintained by the Office. Applicants traverse the rejection for the following reasons.

In the previous "Response to Office Action" dated December 20, 2002, applicants argued the Adachi reference does not teach or suggest a composition or method involving the use of the composition, wherein the composition comprises a PQQ-dependent glucose dehydrogenase with an enzyme activity that is 100 to 2000 kU per gram of the total components. Although the Adachi reference does not specify any specific activity of the PQQ-dependent glucose dehydrogenase, applicants have determined that the specific activity of the PQQ-dependent glucose dehydrogenase is not within the claimed range of 100 to 2000

kU per gram of the total components. For example, as described in the accompanying Rule 132 Declaration of Seiji Takeshima (see Attachment A), applicants have determined that the glucose dehydrogenase activity relative to the total dry weight in the aqueous composition of Example 4 of the Adachi reference is about 0.167 kU/g. The composition of the pending claims has an enzyme activity of 100 to 2000 kU glucose dehydrogenase/g, which is remarkably higher than that of the Adachi reference (approximately 600 or more times higher). Thus, the Adachi reference does not teach or suggest an enzyme activity within the range recited in the pending claims.

The Office has requested a translation of the Adachi reference, such that the Office can ascertain that the comparison between the enzyme activities of the composition of the present invention and the composition disclosed in the Adachi reference was a side-to-side comparison. As requested by the Office, a translation of selected portions of the Adachi reference (including paragraph [0031] of Example 4 of the Adachi reference) is provided herewith (see Attachment B). As is apparent from the translation of the Adachi reference, all factors are identical between the compositions comprising the enzymes except those resulting in the unexpected results of the present invention. The composition of the present invention comprises (i) a PQQ-dependent GDH, (ii) at least one organic acid selected from glutamic acid and other, specifically recited organic acids, and (iii) albumin. In contrast, the composition of the Adachi reference comprises (i) a PQQ-dependent GDH, (ii) at least one amino acid selected from glutamic acid and similar amino acids, (iii) serum albumin, and (iv) calcium ion or calcium salt (see, e.g., Example 4 of the Adachi reference). Applicants selected Example 4 of the Adachi reference for comparison with the claimed composition because Example 4 of the Adachi reference was the most similar to the claimed composition.

In contrast to the present invention, the composition of the Adachi reference requires calcium as an essential component. The Adachi reference discloses that a high enzyme-stabilizing effect can be achieved by use of the combination of calcium ion and a specific amino acid (see, e.g., paragraph [0009] of the Adachi reference). The composition of the pending claims, however, stably maintains the enzyme activity of PQQ-dependent GDH even in the absence of calcium. Thus, since the Adachi reference discloses that a high enzyme-stabilizing effect only can be obtained in the presence of a calcium ion, the Adachi reference teaches away from the present invention.

Moreover, the Sode reference does not cure the deficiencies of the Adachi reference in the manner necessary to arrive at the present invention. The enzyme composition taught by the Sode reference does not have a PQQ-dependent glucose dehydrogenase activity of 100 to 2000 kU per gram of the total components, nor does the composition contain the stabilizing

compounds recited in the pending claims. Accordingly the Sode and Adachi references does not render the pending claims obvious.

Applicants previously submitted a Rule 132 declaration describing the specific activity of Compositions 4-9 of Example 1, and Compositions 2-3 of Example 2. The Office contends that the Rule 132 declaration is defective because it does not contain the requisite penalty paragraph. The Office also contends that the declaration does not indicate the components of the preparation tested, or whether specific amounts of stabilizers were added to obtain the results of the Examples. Applicants have prepared a new Rule 132 Declaration of Seiji Takeshima (see Attachment C), which contains the penalty paragraph and addresses the Office's concerns. Specifically, the new Rule 132 Declaration includes the weight percent of the stabilizing agents (as described in Tables 1 and 2 of the specification), the enzyme weight percent, and the enzyme activity. The Rule 132 Declaration demonstrates that compositions comprising PQQ-dependent GDH having a specific enzyme activity of about 100-200 kU/g can be obtained according to the present invention.

The Office contends that applicants state that the concentration of stabilizing agents in the composition of the present invention is remarkably low, but the claims, as written, do not recite specific amounts of stabilizers. Claims 1 and 3 recite the specific activity of PQQ-dependent GDH. The specific activity is a proportion of the enzyme activity based on the total weight of the composition. As such, a composition with the high specific activity recited in the claims signifies that the composition weight, and thus the amount of stabilizer, is small per enzyme activity unit. The relatively small required amount of stabilizer per enzyme activity unit results in the advantages of the present invention, including reduction in errors in the pipetting the composition solution, less impurities that lower the sensitivity of the composition, and suppression of uneven application of the composition in applying the composition to a sensor tip.

The Office contends that applicants' showing of unexpected results is not commensurate in scope with the pending claims. Rather, the Office contends that applicants' showing of unexpected results would only pertain to claims limited to a PQQ-dependent glucose dehydrogenase obtained from *Acinetobacter calcoaceticus* NCIMB 11517. However, PQQ-dependent GDHs from other organisms also would show high specific activities in the compositions and methods of the present invention, independent of the origins of the PQQ-dependent glucose dehydrogenase. This is because the amino acid sequences of glucose dehydrogenases obtained from different sources have a high degree of homology. This high degree of homology of amino acid sequences leads to similarities of higher structures of enzyme proteins, resulting in the similar enzymatic activities. For this

reason, the unexpected results pertain to more than just PQQ-dependent glucose dehydrogenases obtained from *Acinetobacter calcoaceticus* NCIMB 11517. For example, applicants have performed an alignment analysis between the amino acid sequence of PQQ-dependent GDH from *Acinetobacter calcoaceticus* LMD79.417 and the amino acid sequence of PQQ-dependent GDH from *Acinetobacter calcoaceticus* NCIMB 11517 (renamed *Acinetobacter baumannii NCIMB* 11517 subsequent to the filing of the present application) recited in the pending claims. The amino acid sequence of PQQ-dependent GDH from *Acinetobacter calcoaceticus* LMD79.417 has 455 amino acids, and the amino acid sequence of PQQ-dependent GDH from *Acinetobacter calcoaceticus* NCIMB 11517 has 456 amino acids.

The results of the alignment analysis (Attachment D) indicate that the amino acid sequences of the two strains show 93.4% homology. Moreover, the amino acids corresponding to the PQQ-binding site, calcium (Ca)-binding site, and active center are identical between the two strains (see table below).

	GDH from LMD79.417	**GDH from NCIMB 11517**
PQQ-binding site	D424	D425
	R406	R407
	R408	R409
	K377	K379
	N229	N229
	R228	R228
Ca-binding site	T348	T349
	P248	P248
	G247	G247
Active Center	Y343	Y344
	Q346	W347
	Q168	Q168
	L169	L169
	D143	D143
	Q76	Q76

Due to the similarity of the amino acid sequences of the PQQ-dependent GDH of different organisms, PQQ-dependent GDH of organisms other than *Acinetobactor calcoaceticus* (subsequently renamed *Acinetobacter baumannii*) NCIMB 11517 also can be

stabilized in the composition of the present invention. Therefore, the effect of the invention according to the pending claims is supported by the specification, especially the Examples, of the present application showing the stabilization of the PQQ-dependent GDH from *Acinetobactor calcoaceticus* (subsequently renamed *Acinetobacter baumannii*) NCIMB 11517.

In summary, the Adachi and Sode references do not teach or suggest an enzyme with the PQQ-dependent glucose dehydrogenase activity of 100 to 2000 kU per gram as required by the pending claims. Accordingly, the Adachi and Sodi references would not have led one of ordinary skill in the art to the PQQ-dependent glucose dehydrogenase composition and method of producing the composition as recited in the pending claims. Therefore, the obviousness rejection of the pending claims should be withdrawn.

Conclusion

The application is considered to be in good and proper form for allowance, and the Examiner is respectfully requested to pass this application to issue. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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